



# Urinary Concentrations of Parabens and Other Antimicrobial Chemicals and Their Association with Couples' Fecundity

Melissa M. Smarr, Rajeshwari Sundaram, Masato Honda, Kurunthachalam Kannan, and Germaine M. Buck Louis

<http://dx.doi.org/10.1289/EHP189>

Received: 10 December 2015

Revised: 22 March 2016

Accepted: 23 May 2016

Published: 10 June 2016

**Note to readers with disabilities:** *EHP* will provide a [508-conformant](#) version of this article upon final publication. If you require a 508-conformant version before then, please contact [ehp508@niehs.nih.gov](mailto:ehp508@niehs.nih.gov). Our staff will work with you to assess and meet your accessibility needs within 3 working days.



National Institute of  
Environmental Health Sciences

## Urinary Concentrations of Parabens and Other Antimicrobial Chemicals and Their Association with Couples' Fecundity

Melissa M. Smarr,<sup>1</sup> Rajeshwari Sundaram,<sup>2</sup> Masato Honda,<sup>3</sup> Kurunthachalam Kannan,<sup>3</sup> and  
Germaine M. Buck Louis<sup>1</sup>

<sup>1</sup>Office of the Director, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Rockville, Maryland, USA; <sup>2</sup>Biostatistics and Bioinformatics Branch, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, Rockville, Maryland, USA;

<sup>3</sup>Wadsworth Center, New York State Department of Health, and Department of Environmental Health Sciences, School of Public Health, State University of New York at Albany, Albany, New York, USA

**Address correspondence to** Melissa M. Smarr, Division of Intramural Population Health Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, 6100 Executive Blvd., Room 7B05, Rockville, MD 20852 USA. Telephone: (301) 435-1118. E-Mail: [melissa.smarr@mail.nih.gov](mailto:melissa.smarr@mail.nih.gov)

**Running Title:** Antimicrobials and Couples' Fecundity

**Acknowledgments:** Funded by the National Institutes of Health, Intramural Research Program, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (contracts N01-HD-3-3355; N01-HD-3-3356; NOH-HD-3-3358; HHSN27500001; HHSN27500003).

**Competing Financial Interests:** The authors declare they have no actual or potential competing financial interests.

## Abstract

**Background:** Human exposure to parabens and other antimicrobial chemicals is continual and pervasive. The hormone disrupting properties of these environmental chemicals may adversely affect human reproduction.

**Objective:** To prospectively assess couples' urinary concentrations of antimicrobial chemicals in the context of fecundity, measured as time-to-pregnancy (TTP).

**Methods:** In a prospective cohort of 501 couples, we examined preconception urinary chemical concentrations of parabens, triclosan and triclorcarban in relation to TTP; chemical concentrations were modeled both continuously and in quartiles. Cox's proportional odds models for discrete survival time were used to estimate fecundability odds ratios (FORs) and 95% confidence intervals (CIs) adjusting for *a priori* defined confounders. In light of TTP being a couple-dependent outcome, both partner and couple-based exposure models were analyzed. In all models, FOR estimates < 1.0 denote diminished fecundity (longer TTP).

**Results:** Overall, 347 (69%) couples became pregnant. The highest quartile of female urinary methyl paraben (MP) concentrations relative to the lowest reflected a 34% reduction in fecundity [aFOR= 0.66; 95% CI=(0.45, 0.97)] and remained so when accounting for couples' concentrations [aFOR= 0.63; 95% CI=(0.41, 0.96)]. Similar associations were observed between ethyl paraben (EP) and couple fecundity for both partner and couple-based-models (p-trend=0.02 and p-trend=0.05, respectively). No associations were observed with couple fecundity when chemicals were modeled continuously.

**Conclusions:** Higher quartiles of preconception urinary concentrations of MP and EP among female partners were associated with reduced couple fecundity in partner-specific and couple-based exposure models.

## **Introduction**

Antimicrobial agents destroy or inhibit the growth and spread of microorganisms such as bacteria and fungi. Human exposure to antimicrobial agents can be attributed to the use of personal care and food products (Chemistry 2005; Sung et al. 2013). Specifically, parabens are a class of non-persistent endocrine disrupting chemicals (EDCs) with antimicrobial properties, found in a variety of commercial products including, pharmaceuticals, nutritional supplements, personal care products and food items (Laura E. Dodge et al. 2015; Guo and Kannan 2013; Liao et al. 2013; Liao and Kannan 2014; Schettler 2006). Additionally, antibacterial agents, triclosan (TCS) and triclocarban (TCC), have widespread commercial application as additives in personal care products, textiles and plastic kitchenware (FDA 2010; Liao and Kannan, 2014b; National Library of Medicine 2013; Gao and Cranston 2008)(Yuan Gao and Cranston 2008)(Yuan Gao and Cranston 2008). The growing use of just antibacterial household products is evident in the increase from a few dozen to over 700 consumer products on the market; the U.S. alone is estimated to reach 1.6 billion dollars in sales by the year 2017 (Levy 2001; Smith 2013; Tan et al. 2002). Specific to personal care products, the assessment of 395 soaps sold in a national chain store and regional grocery store in the U.S. found antibacterial agents to be present in 76% of liquid soaps and 29% of bar soaps (Perencevich et al. 2001).

Biomonitoring data underscore the ubiquitous prevalence of these environmental chemicals, with 99%, 92% and 75% of the U.S. general population having detectable concentrations of methyl paraben (MP)propyl paraben (PP), and TCS in urine, respectively (Calafat et al. 2010). These data also reported gender differences in estimated paraben exposure but not TCS; female urinary MP and PP concentrations were higher than those found in males. Parabens, having weak

estrogenic properties in comparison with estradiol, possess an affinity for the estrogen receptor in a manner directly related with the size of the alkyl group on the paraben (Witorsch and Thomas 2010). The reproductive toxicity of these antimicrobial chemicals has primarily been demonstrated in rodent models. In one study, daily subcutaneous exposure to parabens among 55 female neonatal rats resulted in abnormalities in ovarian folliculogenesis – increased primordial follicles and decreased early primary follicles (Ahn et al. 2012). Another study of oral exposure to parabens among prepubertal female rats found decreased ovarian weight and histopathological abnormalities in the ovaries, among other adverse effects (Vo et al. 2010). On the male side, rats aged at 19-21 days were exposed to butyl paraben (BP) or PP via diet for eight weeks were found to have reduced secretion of testosterone and sperm production (Oishi 2001, 2002). Albeit scarce, epidemiological findings suggest associations between paraben exposure and ovarian aging, sperm DNA damage, and reduced fertility (Dodge et al. 2015; Meeker et al. 2011; Smith et al. 2013). Nevertheless, studies of preconception urinary levels of antimicrobial chemicals (i.e., parabens, TCS and TCC) and prospectively assessed couple fecundity, as measured by time-to-pregnancy (TTP) or the number of menstrual cycles required to achieve pregnancy, are lacking; therefore serving as the motivation for this study.

## **Materials and Methods**

### *Study population*

The Longitudinal Investigation of Fertility and the Environment (LIFE) study population comprised 501 reproductive aged couples who were recruited from 16 counties in Michigan and Texas between 2005 and 2009. Couples were recruited upon discontinuing contraception for the purpose of becoming pregnant, as previously described (Buck Louis et al. 2011). Inclusion criteria were minimal: females aged 18-40 and males  $\geq 18$  years; in a committed relationship and

planning to try for pregnancy or currently off contraception for  $\leq 2$  months; females had to have menstrual cycles between 21-42 days without any injectable hormonal contraceptives in the past year; and an ability to communicate in English or Spanish. Institutional review board approvals were obtained from all collaborating institutions; couples gave written informed consent prior to study participation and any data collection.

### *Biospecimen collection and analysis*

During the enrollment home visit, each partner of the couple provided a spot urine and non-fasting blood sample. Using established protocols (Asimakopoulos et al. 2014), MP, EP, PP, BP, benzyl paraben (BzP), heptyl paraben (HP), 4-hydroxy benzoic acid (4-HB), 3,4-dihydroxy benzoic acid (3,4-DHB), methyl-protocatechuic acid (OH-MeP), and ethyl-protocatechuic acid (OH-EtP) were quantified along with TCS and TCC by the Wadsworth Center, New York State Department of Health. Specifically, 300  $\mu$ L of 1 M ammonium acetate containing 30 U of  $\beta$ -glucuronidase (pH=5.5) was added to 500  $\mu$ L of urine sample, followed by incubation at 37 °C for 12 hours. Target analytes were extracted three times with ethyl acetate and were quantified as nanograms/milliliter (ng/mL) by ultra-performance liquid chromatography (Acquity I Class; Waters, Milford, MA) coupled with an electrospray triple quadrupole tandem mass spectrometry (API 5500; AB SCIEX, Framingham, MA) (UPLC-ESI-MS/MS); separation of target analytes was carried by a Kinetex C18 (1.3 $\mu$ , 100A, 50  $\times$  2.1 mm) column (Phenomenex; Torrance, CA) with a SecurityGuard<sup>®</sup> guard column (Phenomenex; Torrance, CA). Quality assurance and quality control parameters included procedural blanks, matrix spikes and duplicate analysis of samples. Labeled internal standards were spiked into all samples and quantification was by isotope dilution. Creatinine was quantified using a Roche/Hitachi Model 912 clinical analyzer

(Dallas, TX) and the Creatinine Plus Assay. In addition, non-fasting blood samples were collected from each partner to measure serum cotinine using liquid chromatography-isotope dilution tandem mass spectrometry and as reported in ng/mL (Bernert et al. 1997).

### *Assessment of lifestyle and couple fecundity*

To capture couples' lifestyles and reproductive health histories, interviews were conducted followed by standard anthropometric assessments to measure body mass index (BMI) (Lohman et al. 1988). Couples completed daily journals about lifestyle and women recorded menstruation and pregnancy results. Female partners were instructed in the use of the Clearblue® Easy home fertility monitor, which tracks estrone-3-glucuronide (E<sub>3</sub>G) and luteinizing hormone (LH) allowing couples to time intercourse relative to peak fertility, which is a proxy marker of ovulation (Behre et al. 2000). Lastly, female partners used the Clearblue® Digital home pregnancy test, capable of detecting 25 mIU/mL human chorionic gonadotropin (hCG), to test for pregnancy on the day of expected menses (Johnson et al. 2015). Therefore, it was possible to differentiate between couples achieving pregnancy in the first few weeks post-enrollment (TTP=0 completed cycles) and those achieving pregnancy during the first fully observed menstrual cycle (TTP=1). Couples were followed up to 12 months of trying at which point TTP was censored.

### *Statistical analysis*

Univariate analyses were performed to assess all chemical distributions and relevant covariates. Female and male partners' lifestyle characteristics were compared using independent t-tests and Chi-square tests for continuous and categorical covariates, respectively. Urinary creatinine and chemical concentrations were natural-log transformed (x+1) to normalize distributions. The

correlation between male and female partner's chemical concentrations was examined with the use of Spearman rank analyses. Median and accompanying interquartile ranges (IQRs) of preconception urinary chemical concentrations were calculated; medians were compared between female and male partners using Wilcoxon-Mann-Whitney tests. To avoid biasing point estimates when assessing health outcomes, the unadjusted ln-transformed instrument measured values for all chemicals were used in statistical models and ln-transformed urinary creatinine was included as a covariate. (Lubin et al. 2004; Richardson and Ciampi 2003; Schisterman et al. 2006). HP was not detected in any urine samples. For BzP and TCC, >80% of urine samples had concentrations below the LOQ prompting us to model them as above/below LOQ. These chemicals were excluded from continuous linear models; point estimates and p-values for dichotomous variables are presented for models of dichotomous urine concentrations. Missing female (6%) and male (12%) urinary chemicals stemming from insufficient urine volume for chemical quantification, resulting from previous use of samples, and missing covariate data (2%) were imputed using Markov Chain Monte Carlo methods (Schafer 1997) to minimize bias (Desai et al. 2011; White and Carlin 2010). Imputations were performed under the assumption that missing urine was not dependent upon couples' TTP and were therefore assumed to be missing at random. Box-Cox transformation was performed on imputed values to achieve normality in the highly skewed imputed values (Osborne 2010). We defined statistical significance as a two-sided p-value < 0.05.

Cox proportional odds models for discrete survival time and allowing for a cycle-varying intercept were used to estimate fecundability odds ratios (FORs) and 95% CIs (Cox 1972). We also accounted for left truncation or time couples were off contraception at enrollment, and right censoring due to attrition. FORs estimate the odds of pregnancy each cycle conditional on not

being pregnant in the previous cycle per unit of chemical change. FOR estimates  $< 1.0$  reflect diminished fecundity (longer TTP), while FORs  $> 1$  reflect enhanced fecundity (shorter TTP).

We first modeled each partner's chemical concentrations then jointly modeled both partners' concentrations in keeping with the couple dependent nature of human reproduction. We *a priori* defined confounders as: age (years), body mass index ( $\text{kg}/\text{m}^2$ ) categorized as [normal ( $<25$ ), overweight (25-30), obese (30-35) and morbidly obese ( $>35$ )],  $\ln(\text{creatinine (mg/dL)})$ , active preconception smoking (serum cotinine above/below 10 ng/mL (Benowitz et al. 2009; Hukkanen et al. 2005)), race/ethnicity (Non-White/White), and household income (above/below \$70,000) (Augood et al. 1998; Calafat et al. 2008; Calafat et al. 2010; Curtin et al. 2015; Dunson et al. 2002; Hassan and Killick 2004; Martin et al. 2015; Ramlau-Hansen et al. 2007; Smith et al. 2012). Fecundity models were first run with continuous urinary concentrations to assess potential linear associations between urinary chemicals and fecundity then in quartiles to assess non-linear relationships. Wald tests were performed to test for linear trend across quartiles of chemical concentrations.

In light of the exploratory nature of this analysis, we undertook sensitivity analysis to assess the robustness of our findings. First, we repeated the above analyses restricting to couples without imputed chemical data, to assess introduction of bias by imputation methods. Next, to examine the role of time with respect to urinary chemical assessment and TTP, models included in the primary analysis were performed restricting the data to include couples with a TTP  $< 2$  cycles. All analyses were performed using SAS software (version 9.4; SAS Institute, Cary, NC)

## Results

Among the 501 enrolled couples, 347 (69%) had an observed human chorionic gonadotropin confirmed pregnancy. The women and men in LIFE Study were primarily college educated (95% and 91%, respectively) and predominantly of non-Hispanic White ethnicity and race (79%). The mean female and male ages were  $30.0 \pm 4.1$  and  $31.8 \pm 4.9$  years, respectively (Table 1). The prevalence of cigarette smoking prior to conception was lower among females than male partners (12%, and 22%, respectively).

The median and accompanying IQRs for creatinine-adjusted urinary concentrations of parabens and their metabolites, and the antibacterial agents, TCS and TCC, for each partner are displayed in Table 2. Generally, parabens and antibacterial chemical concentrations were readily detectable in all couples, ranging from 78%-100% of concentrations >LOQ in women and 74%-100% in males. TCS was detected in 93% and 87% of females and males, respectively. Creatinine-adjusted urinary concentrations of most parabens presented in Table 2 were significantly higher for females than males ( $p < 0.0001$ ); unadjusted chemical concentrations were similar (see Supplemental Material, Table S1).

When modeling partners individually, no associations were observed between ln-transformed chemical concentrations and couple fecundity even after adjustment with the exception of the biomarker 4-HB (Table 3). Increasing concentrations of male partners' urinary 4-HB was marginally associated with enhanced couple fecundity (FOR= 1.17; 95% CI= (1.00, 1.36). No associations were observed when modeling couples' concentrations and fecundity (Table 3).

Figure 1 presents the unadjusted and adjusted FORs for chemicals (in quartiles) found having a significant association with couple fecundity when modeling each partner of the couple.

Females in the 4<sup>th</sup> quartile ( $\geq 104$  ng/ml) of MP concentrations had a 28% reduction in fecundity

that increased to a 34% reduction in adjusted models [FOR=0.72; 95% CI=(0.51,1.03 and aFOR= 0.66; 95% CI=(0.45, 0.97), respectively] when compared to women in the 1<sup>st</sup> quartile (< 12 ng/mL). Moreover, a significant (p=0.02) trend was observed. A similar relationship was observed when comparing females in the highest ( $\geq 5.62$  ng/mL) versus lowest quartile (< 0.27 ng/mL) of EP in both unadjusted and adjusted models [FOR=0.66; 95% CI=(0.47, 0.93) and aFOR= 0.66; 95% CI=(0.46, 0.95), respectively], reflecting in a significant trend (p=0.02). No association was observed between the remaining parabens and couple fecundity when modeled in quartiles (see Supplemental Material, Table S2). With regard to male partners' concentrations, significant FORs were observed for BP and 4-HB but only in the unadjusted analysis (Figure 1). No significant associations were observed for any of the remaining parabens, as quantified in men (See Supplemental Material, Table S2).

When modeling both partners' concentrations given the low correlations between their concentrations (ranging from  $r = -0.07$  to 0.10, Table 2), female partners' MP and EP concentrations remained significantly associated with diminished fecundity by 37% (aFOR=0.63; 95% CI= (0.41, 0.96) and 33% (aFOR=0.67; 95% CI= (0.46, 0.98), respectively after adjusting for the male partners' concentrations (Figure 2). Findings not achieving significance in the couple based model are presented in Supplemental Material, Table S3.

## Discussion

In the first prospective assessment of couples' fecundity in the context of preconception urinary antimicrobial chemicals, we found little evidence that parabens, TCS and TCC were associated with couple fecundity with in models of ln-transformed chemical concentrations. However, a 34% reduction in couple fecundity was observed for female partners with urinary MP concentrations in the highest quartile and urinary EP concentrations in the third quartile,

compared with the MP concentrations < 12 ng/mL and EP concentrations < 0.27 ng/mL, after adjustment. Likewise, we observed a 37% and 33% reduction in couple fecundity for female MP and EP concentrations when accounting for both partners' urinary chemical concentration in covariate-adjusted analyses. In LIFE, median antimicrobial concentrations were approximately 10 times higher for female than male partners. Median urinary MP concentrations for females (60.0 µg/g creatinine) and males (6.37 µg/g creatinine) in the LIFE Study were generally lower than those reported in the NHANES cross-sectional survey (147 µg/g and 21.1 µg/g creatinine, respectively) (Calafat et al. 2008). These findings suggest that for couple fecundity to be negatively associated with preconception urinary MP and EP, the female partner's concentration needs to be at the higher end of the distribution.

Comparison of our findings to previous work is limited considering that only a few epidemiological studies have focused on non-persistent environmental chemicals as it relates to human fecundity (Velez et al. 2015a, b), and even fewer have included both partners despite TTP being a couple dependent outcome (Buck Louis et al. 2014a, b; Specht et al. 2015). We are unaware of any previous work on preconception exposure to antimicrobial chemicals (i.e., parabens, triclosan) in relation to couple fecundity measured as TTP, precluding a more complete interpretation of our findings. Nonetheless, while not directly comparable, in a previous study assessing triclosan and fecundity as measured by retrospectively reported TTP in pregnant women, a 14% reduction in fecundity was reported among women in the highest (>72 ng/mL) versus lowest quartile of triclosan (TCS) (Velez et al. 2015a); a finding that our analysis did not corroborate.

Still, our results are strengthened by several components of the LIFE cohort study design as well as analytic methods. Primarily the prospective assessment of TTP is a sensitive measure of

couple fecundity. Women in LIFE were screened for pregnancy at study enrollment with the use of the Clearblue<sup>®</sup> Digital home pregnancy test, capable of detecting pregnancy with 99% accuracy (false positive results range 0-0.3%, depending on pregnancy test lot) when used from the day of expected menstrual cycle (Tomlinson et al. 2008). Moreover, the use of digital pregnancy tests removes ambiguity in the interpretation of test results based on color and symbol. Women without a positive hCG test at enrollment in the study were eligible to participate in the cohort; ensuring the accurate capture of prospectively assessed TTP. Additionally, predictors of estimated antimicrobial exposure were thoughtfully considered in the present analysis. Potential chemical associations with couple fecundity were explored with ln-transformed urinary concentrations and then by categorizing urinary chemical concentrations to assess potential linear and nonlinear relationships. Also, we explored models of unadjusted chemical concentrations to avoid the potential bias induced by using creatinine-adjusted chemical concentrations in studies of human health (Cocker et al. 2011; Weaver et al. 2016); all models were also performed on non-imputed data resulting in marginally significant findings that did not remain in the imputation analysis. Furthermore, our models included the novel quantification of hydroxylated metabolites of MP (OH-MeP) and EP (OH-EtP), and non-specific paraben biomarkers 4-HB and 3,4-DHB (Wang and Kannan 2013) as predictors of estimated exposures in relation to couple fecundity. Of note is the observation that the measured concentrations of these metabolites were much higher than those of the parent parabens, highlighting the stability of such compounds and usefulness as exposure biomarkers (Wang and Kannan 2013). Despite the use of such novel biomarkers, our findings are limited by reliance on a single spot urine collected at enrollment given the short biologic half-lives of parabens, TCS and TCC (Sandborgh-Englund et al. 2006; Soni et al. 2005). Still, serial measurements of

parabens and TCS over a period of several months have been reported to have relatively high correlation reflecting continual exposure, i.e., ICCs ranging from 0.40 to 0.65 in non-pregnant adult females and males of reproductive age from the USA (Massachusetts), Belgian and Danish populations (Dewalque et al. 2015; Lassen et al. 2013). Therefore, since 90% of pregnancies in the LIFE Study occurred within the first six menstrual cycles and 38% within cycles 0-1 (Buck Louis et al. 2012), the potential for exposure misclassification may be reduced but not eliminated. We attempted to evaluate this consideration by restricting our analysis to couples with a TTP < 2 cycles (n=167) and continued to observe a diminished fecundity for the women in the highest vs. lowest quartile of MP (0.59; 95% CI= 0.20, 1.70). Furthermore, our findings were generally robust when we restricted our analysis to only include those couples with measured chemical concentrations.

Despite the paucity of data in the context of biologic plausibility, given the robust, significant associations observed between female MP and EP and couple fecundity in our cohort, potential mechanisms have been considered. Primarily, the estrogenic activity of parabens has largely been established in animal studies (Boberg et al. 2010; Darbre and Harvey 2008; Karpuzoglu et al. 2013). Although animal studies have demonstrated MP and EP to be less estrogenic than PP and BP (Vo et al. 2010; Witorsch and Thomas 2010), we observed significant associations between higher female concentrations of MP and EP in urine and couple fecundity.

Furthermore, the observed relationship between female urinary concentrations of MP and EP and couple fecundity in our analysis may, in part, be explained by other mechanisms. Oxidative stress is a purported factor of female reproductive disorders including endometriosis and polycystic ovary syndrome, which have implications for reduced fecundity (Agarwal et al. 2012; Ruder et al. 2008). In general, parabens have been correlated with the urinary biomarker of

oxidative stress, 8-hydroxy-2'-deoxyguanosine in humans (Asimakopoulos et al. 2015), while MP and EP have are suspected of reacting with oxygen in the skin to produce a free radical or reactive oxygenated species (Nishizawa et al. 2006). Also, high concentrations of MP were measured in female than male urine samples in the LIFE study, which may be reflective of a greater use of personal care products among female partners (Manová et al. 2013; Wu et al. 2010); therefore, partly explaining our urinary MP and couple fecundity findings. However, we are also aware of the potential for chance findings, having performed multiple comparisons in the current analysis.

The lack of association between male antimicrobial concentrations and couple fecundity may be explained by the relatively low urinary concentrations of parabens, TCS and TCC measured among males in our cohort. Therefore we were unable to corroborate the findings of a previous *in vitro* assessment which demonstrated anti-androgenic effects of several parabens and TCS in response to large micro molar concentrations with the use of a cell-based human androgen receptor-mediated bioassay (Chen et al. 2007). Additionally we recognize that our models did not adjust for semen quality. While semen quality is an important factor of couple fecundity, to avoid model overadjustment (Schisterman, 2009) we decided against the adjustment for semen quality in the present analysis given the lack of an association between any of the 35 semen quality parameters and TTP in our study cohort in a previous analysis (Buck Louis et al. 2014).

In light of the observational nature of this work, the absence of longitudinal chemical measurements and residual confounding, cautious interpretation of our findings is warranted. Our results await corroboration by larger prospective cohort studies of repeated preconception urinary measures of parabens and other antimicrobial chemicals in the context of couple fecundity.

## **Conclusion**

Female but not male partners' preconception urinary concentration of MP and EP were associated with a 37% and 33% reduction in couple fecundity, as measured by a longer TTP.

## References

- Agarwal A, Aponte-Mellado A, Premkumar BJ, Shaman A, Gupta S. 2012. The effects of oxidative stress on female reproduction: A review. *Reproductive Biology and Endocrinology* 10:1-31.
- Ahn H-J, An B-S, Jung E-M, Yang H, Choi K-C, Jeung E-B. 2012. Parabens inhibit the early phase of folliculogenesis and steroidogenesis in the ovaries of neonatal rats. *Molecular Reproduction and Development* 79:626-636.
- Asimakopoulos AG, Wang L, Thomaidis NS, Kannan K. 2014. A multi-class bioanalytical methodology for the determination of bisphenol a diglycidyl ethers, p-hydroxybenzoic acid esters, benzophenone-type ultraviolet filters, triclosan, and triclocarban in human urine by liquid chromatography-tandem mass spectrometry. *Journal of chromatography A* 1324:141-148.
- Asimakopoulos AG, Xue J, De Carvalho BP, Iyer A, Abualnaja KO, Yaghmoor SS, et al. Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in jeddah, saudi arabia. *Environmental research*. 2015. doi: 10.1016/j.envres.2015.11.029. [Epub ahead of print]
- Behre HM, Kuhlage J, Gassner C, Sonntag B, Schem C, Schneider HP, et al. 2000. Prediction of ovulation by urinary hormone measurements with the home use clearplan fertility monitor: Comparison with transvaginal ultrasound scans and serum hormone measurements. *Human reproduction* 15:2478-2482.
- Benowitz NL, Bernert JT, Caraballo RS, Holiday DB, and Wang J. Optimal serum cotinine levels for distinguishing cigarette smokers and nonsmokers within different racial/ethnic groups in the United States between 1999 and 2004. *Am J Epidemiol*. 2009;169:236-248.
- Bernert JT, Jr., Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. 1997. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. *Clinical chemistry* 43:2281-2291.
- Boberg J, Taxvig C, Christiansen S, Hass U. 2010. Possible endocrine disrupting effects of parabens and their metabolites. *Reproductive toxicology* 30:301-312.
- Buck Louis GM, Schisterman EF, Sweeney AM, Wilcosky TC, Gore-Langton RE, Lynch CD, et al. 2011. Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development--the life study. *Paediatric and perinatal epidemiology* 25:413-424.
- Buck Louis GM, Sundaram R, Schisterman EF, Sweeney AM, Lynch CD, Gore-Langton RE, et al. 2012. Heavy metals and couple fecundity, the life study. *Chemosphere* 87:1201-1207.
- Buck Louis GM, Kannan K, Sapra KJ, Maisog J, Sundaram R. 2014. Urinary concentrations of benzophenone-type ultraviolet radiation filters and couples' fecundity. *American journal of epidemiology*.

- Buck Louis GM, Sundaram R, Sweeney AM, Schisterman EF, Maisog J, Kannan K. 2014. Urinary bisphenol a, phthalates, and couple fecundity: The longitudinal investigation of fertility and the environment (life) study. *Fertility and sterility* 101:1359-1366.
- Buck Louis GM, Sundaram R, Schisterman EF, Sweeney A, Lynch CD, Kim S, et al. 2014. Semen quality and time to pregnancy: The longitudinal investigation of fertility and the environment study. *Fertility and sterility* 101:453-462.
- Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. 2008. Urinary concentrations of triclosan in the u.S. Population: 2003–2004. *Environmental health perspectives* 116:303-307.
- Calafat AM, Ye X, Wong LY, Bishop AM, Needham LL. 2010. Urinary concentrations of four parabens in the u.S. Population: Nhanes 2005–2006. *Environmental health perspectives* 118:679-685.
- Chen J, Ahn KC, Gee NA, Gee SJ, Hammock BD, Lasley BL. 2007. Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. *Toxicology and Applied Pharmacology* 221:278-284.
- Cox DR. 1972. Regression models and life-tables. *Journal of the Royal Statistical Society Series B (Methodological)* 34:187-220.
- Cocker J, Mason HJ, Warren ND, Cotton RJ. 2011. Creatinine adjustment of biological monitoring results. *Occupational Medicine* 61:349-353.
- Darbre PD, Harvey PW. 2008. Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. *Journal of applied toxicology : JAT* 28:561-578.
- Desai M, Esserman DA, Gammon MD, Terry MB. 2011. The use of complete-case and multiple imputation-based analyses in molecular epidemiology studies that assess interaction effects. *Epidemiologic perspectives & innovations : EP+I* 8:5.
- Dewalque L, Pirard C, Vandepaer S, Charlier C. 2015. Temporal variability of urinary concentrations of phthalate metabolites, parabens and benzophenone-3 in a belgian adult population. *Environmental research* 142:414-423.
- Dodge LE, Kelley KE, Williams PL, Williams MA, Hernández-Díaz S, Missmer SA, et al. 2015. Medications as a source of paraben exposure. *Reproductive toxicology* 52:93-100.
- Dodge LE, Williams PL, Williams MA, Missmer SA, Toth TL, Calafat AM, et al. 2015. Paternal urinary concentrations of parabens and other phenols in relation to reproductive outcomes among couples from a fertility clinic. *Environmental health perspectives*.
- Guo Y, Kannan K. 2013. A survey of phthalates and parabens in personal care products from the united states and its implications for human exposure. *Environmental science & technology* 47:14442-14449.

- Hukkanen J, Jacob P, Benowitz NL. 2005. Metabolism and disposition kinetics of nicotine. *Pharmacological Reviews* 57:79-115.
- Johnson S, Cushion M, Bond S, Godbert S, Pike J. Comparison of analytical sensitivity and women's interpretation of home pregnancy tests. *Clin Chem Lab Med* 2015;53:391-402.
- Lassen TH, Frederiksen H, Jensen TK, Petersen JH, Main KM, Skakkebaek NE, et al. 2013. Temporal variability in urinary excretion of bisphenol a and seven other phenols in spot, morning, and 24-h urine samples. *Environmental research* 126:164-170.
- Levy SB. 2001. Antibacterial household products: Cause for concern. *Emerging infectious diseases* 7:512-515.
- Liao C, Liu F, Kannan K. 2013. Occurrence of and dietary exposure to parabens in foodstuffs from the united states. *Environmental science & technology* 47:3918-3925.
- Liao C, Kannan K. 2014. Concentrations and composition profiles of parabens in currency bills and paper products including sanitary wipes. *The Science of the total environment* 475:8-15.
- Lohman TG, Roche AF, Martorell R. 1988. Anthropometric standardization reference manual. Champaign, IL:Human Kinetics Books.
- Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. *Environmental health perspectives* 112:1691-1696.
- Manová E, von Goetz N, Keller C, Siegrist M, Hungerbühler K. 2013. Use patterns of leave-on personal care products among swiss-german children, adolescents, and adults. *International Journal of Environmental Research and Public Health* 10:2778-2798.
- Meeker JD, Yang T, Ye X, Calafat AM, Hauser R. 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. *Environmental health perspectives* 119:252-257.
- Nishizawa C, Takeshita K, Ueda J, Nakanishi I, Suzuki KT, Ozawa T. 2006. Reaction of para-hydroxybenzoic acid esters with singlet oxygen in the presence of glutathione produces glutathione conjugates of hydroquinone, potent inducers of oxidative stress. *Free radical research* 40:233-240.
- Oishi S. 2001. Effects of butylparaben on the male reproductive system in rats. *Toxicology and industrial health* 17:31-39.
- Oishi S. 2002. Effects of propyl paraben on the male reproductive system. *Food and Chemical Toxicology* 40:1807-1813.
- Perencevich EN, Wong MT, Harris AD. 2001. National and regional assessment of the antibacterial soap market: A step toward determining the impact of prevalent antibacterial soaps. *American journal of infection control* 29:281-283.

- Richardson DB, Ciampi A. 2003. Effects of exposure measurement error when an exposure variable is constrained by a lower limit. *American journal of epidemiology* 157:355-363.
- Ruder EH, Hartman TJ, Blumberg J, Goldman MB. 2008. Oxidative stress and antioxidants: Exposure and impact on female fertility. *Human reproduction update* 14:345-357.
- Sandborgh-Englund G, Adolfsson-Erici M, Odham G, Ekstrand J. 2006. Pharmacokinetics of triclosan following oral ingestion in humans. *Journal of toxicology and environmental health Part A* 69:1861-1873.
- Schafer J. 1997. *Analysis of incomplete multivariate data*. New York:Chapman and Hall.
- Schettler T. 2006. Human exposure to phthalates via consumer products. *International journal of andrology* 29:134-139; discussion 181-135.
- Schisterman EF, Vexler A, Whitcomb BW, Liu A. 2006. The limitations due to exposure detection limits for regression models. *American journal of epidemiology* 163:374-383.
- Schisterman EF, Cole SR, Platt RW. 2009. Overadjustment bias and unnecessary adjustment in epidemiologic studies. *Epidemiology* 20:488-495.
- Smith KW, Souter I, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, et al. 2013. Urinary paraben concentrations and ovarian aging among women from a fertility center. *Environmental health perspectives* 121:1299-1305.
- Smith S. 2013. U.S. Disinfectant & antimicrobial chemicals market. Available: <http://www.prweb.com/releases/2013/9/prweb11150188.htm>
- Soni MG, Carabin IG, Burdock GA. 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food and Chemical Toxicology* 43:985-1015.
- Specht IO, Bonde JP, Toft G, Lindh CH, Jonsson BA, Jorgensen KT. 2015. Serum phthalate levels and time to pregnancy in couples from greenland, poland and ukraine. *PloS one* 10:e0120070.
- Sung S-Y, Sin LT, Tee T-T, Bee S-T, Rahmat AR, Rahman WAWA, et al. 2013. Antimicrobial agents for food packaging applications. *Trends in Food Science & Technology* 33:110-123.
- Tan L, Nielsen NH, Young DC, Trizna Z. 2002. Use of antimicrobial agents in consumer products. *Archives of dermatology* 138:1082-1086.
- Tomlinson C, Marshall J, Ellis JE. 2008. Comparison of accuracy and certainty of results of six home pregnancy tests available over-the-counter. *Current Medical Research and Opinion* 24:1645-1649.
- Van Nostrand's Encyclopedia of Chemistry. 2005. Antimicrobial agents (foods). In: Van Nostrand's Encyclopedia of Chemistry:John Wiley & Sons, Inc.

- Velez MP, Arbuckle TE, Fraser WD. 2015a. Female exposure to phenols and phthalates and time to pregnancy: The maternal-infant research on environmental chemicals (mirec) study. *Fertility and sterility* 103:1011-1020.e1012.
- Velez MP, Arbuckle TE, Fraser WD. 2015b. Maternal exposure to perfluorinated chemicals and reduced fecundity: The mirec study. *Human reproduction* 30:701-709.
- Vo TT, Yoo YM, Choi KC, Jeung EB. 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. *Reproductive toxicology* 29:306-316.
- Wang L, Kannan K. 2013. Alkyl protocatechuates as novel urinary biomarkers of exposure to p-hydroxybenzoic acid esters (parabens). *Environment international* 59:27-32.
- Weaver VM, Kotchmar DJ, Fadrowski JJ, Silbergeld EK. 2016. Challenges for environmental epidemiology research: Are biomarker concentrations altered by kidney function or urine concentration adjustment[quest]. *J Expos Sci Environ Epidemiol* 26:1-8.
- White IR, Carlin JB. 2010. Bias and efficiency of multiple imputation compared with complete-case analysis for missing covariate values. *Statistics in medicine* 29:2920-2931.
- Witorsch RJ, Thomas JA. 2010. Personal care products and endocrine disruption: A critical review of the literature. *Critical reviews in toxicology* 40 Suppl 3:1-30.
- Wu XM, Bennett DH, Ritz B, Cassady DL, Lee K, Hertz-Picciotto I. 2010. Usage pattern of personal care products in california households. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association* 48:3109-3119.
- Yuan Gao, Cranston R. 2008. Recent advances in antimicrobial treatments of textiles. *Textile Research Journal* 78:60-72.

**Table 1.** Comparison of partners by select baseline characteristics, the LIFE Study (n=501).

Characteristics	Females	Males
	n (%)	n (%)
Age at baseline <sup>a</sup> *	30.0 ± 4.1	31.8 ± 4.9
BMI at baseline *		
Under/healthy (BMI < 25)	229 (46)	84 (17)
Overweight (25 ≤ BMI < 30)	136 (27)	206 (42)
Obese (30 ≤ BMI < 35)	66 (13)	131 (26)
Morbidly obese (BMI ≥ 35)	69 (14)	75 (15)
Missing values	1 (<1)	5 (<1)
Mean ± SD*	27.6 ± 7.3	29.8 ± 5.6
Race/Ethnicity		
Non-Hispanic White	393 (79)	394 (79)
Non-Hispanic Black	24 (5)	23 (5)
Hispanic	50 (10)	45 (9)
Other	31 (6)	36 (7)
Missing values	3 (< 1)	3 (< 1)
Household income		
<\$70,000	157 (32)	163 (33)
≥ \$70,000	334 (68)	328 (67)
Missing values	10 (2)	10 (2)
Parity conditional on gravidity		
No prior pregnancy	210 (42)	215 (43)
Prior pregnancy		
Without live birth(s)	53 (11)	44 (9)
With live birth(s)	235 (47)	239 (48)
Missing values	3 (<1)	3 (<1)
Active smoking status*		
Yes (serum cotinine ≥ 10 ng/ml)	58 (12)	106 (22)
No (serum cotinine < 10 ng/ml)	431 (88)	387 (79)
Missing values	12 (2)	9 ( 2)

Abbreviations: SD, standard deviation; BMI, body mass index (kg/m<sup>2</sup>)

<sup>a</sup> Mean ± SD

\*p<0.05;\*\* p<0.0001 from independent t-test for continuous characteristics or chi-square test for categorical characteristics.

**Table 2.** Distribution of urinary creatinine-adjusted antimicrobial phenolic concentrations by partner.

Chemical ( $\mu\text{g/g}$ )	Female (n=470)			Males (n=439)		Partners' Chemical Correlation
	LOQ	%<LOQ <sup>a</sup>	Median (IQR)	%<LOQ <sup>a</sup>	Median (IQR)	
Creatinine <sup>**</sup>	3.5	0	77.9 (35.1, 136)	0	140 (72.3, 201)	0.19 <sup>**</sup>
MP <sup>**</sup>	0.2	0	60.0 (17.8, 154)	1	6.37 (1.84, 25.8)	0.06
EP <sup>**</sup>	0.1	5	1.51 (0.457, 8.97)	18	0.33 (0.13, 1.25)	0.08
PP <sup>**</sup>	0.05	2	19.5 (5.45, 59.7)	5	1.48 (0.426, 5.73)	0.02
BP <sup>**</sup>	0.1	22	0.83 (0.12, 4.26)	70	0.03 (0.01, 0.16)	0.01
BzP <sup>*</sup>	0.1	84	0.02 (0.00, 0.06)	91	0.01 (0.00, 0.04)	0.35 <sup>**</sup>
HP	0.1	100	0.00 (0.00, 0.00)	100	0.00 (0.00, 0.00)	0.04
4-HB <sup>**</sup>	2	0	671 (424, 1138)	0	478 (335, 692)	0.15 <sup>*</sup>
3,4-DHB <sup>**</sup>	5	1	43.2 (27.7, 73.0)	1	26.1 (16.8, 45.9)	0.15 <sup>*</sup>
OH-MeP <sup>**</sup>	1	0	31.4 (14.3, 65.9)	1	18.3 (9.14, 41.7)	0.23 <sup>**</sup>
OH-EtP <sup>**</sup>	1	15	6.46 (1.87, 20.7)	26	2.92 (0.883, 10.6)	0.11 <sup>*</sup>
TCS	2	7	16.8 (5.32, 67.5)	13	16.2 (4.41, 64.4)	0.31 <sup>**</sup>
TCC <sup>*</sup>	0.2	87	0.02 (0.00, 0.06)	88	0.01 (0.00, 0.03)	0.35 <sup>**</sup>

Abbreviations: LOQ, limit of quantification; IQR, interquartile range; MP, methyl paraben; EP, ethyl paraben; PP, propyl paraben; BP, butyl paraben; BzP, benzyl paraben; HP, heptyl paraben; 4-HB, 4-hydroxy benzoic acid; 3,4-DHB, 3,4-dihydroxy benzoic; OH-Me-P, methyl-protocatechuic acid; OH-Et-P, ethyl-protocatechuic acid; TCS, triclosan; TCC, triclocarban.

<sup>a</sup> LOQ for all urinary chemicals; limit of detection (LOD) for creatinine.

\* p<0.05; \*\* p<0.0001, Wilcoxon-Mann-Whitney tests comparing median female and male creatinine-adjusted urinary chemical concentrations.

**Table 3.** Individual partners' urinary antimicrobials and fecundability odds ratios (FORs), n=501.

Chemical	Female Partners			Male Partners		
	Unadjusted FOR (95% CI)	Adjusted <sup>a</sup> FOR (95% CI)	Adjusted <sup>b</sup> FOR (95% CI)	Unadjusted FOR (95% CI)	Adjusted <sup>a</sup> FOR (95% CI)	Adjusted <sup>b</sup> FOR (95% CI)
MP	0.97 (0.87, 1.08)	0.96 (0.85, 1.09)	0.95 (0.83, 1.09)	1.00 (0.96, 1.04)	1.00 (0.96, 1.05)	1.01 (0.96, 1.05)
EP	1.01 (0.97, 1.06)	1.01 (0.97, 1.06)	1.02 (0.97, 1.06)	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)	1.00 (0.96, 1.03)
PP	1.00 (0.97, 1.03)	1.00 (0.97, 1.03)	0.99 (0.96, 1.03)	1.01 (0.99, 1.02)	1.01 (0.99, 1.03)	1.01 (0.99, 1.03)
BP	1.01 (0.96, 1.06)	1.01 (0.95, 1.06)	1.00 (0.94, 1.07)	1.01 (0.98, 1.04)	1.01 (0.99, 1.05)	1.01 (0.98, 1.05)
BzP <sup>d</sup>	1.04 (0.70, 1.55)	0.98 (0.63, 1.52)	1.00 (0.67, 1.50)	1.20 (0.84, 1.70)	1.16 (0.81, 1.67)	1.20 (0.84, 1.71)
4-HB	1.08 (0.95, 1.22)	1.14 (0.96, 1.35)	1.10 (0.93, 1.32)	1.17 (1.00, 1.36) <sup>c</sup>	1.10 (0.90, 1.36)	1.07 (0.87, 1.33)
3,4-DHB	0.98 (0.85, 1.13)	0.99 (0.83, 1.18)	0.99 (0.82, 1.19)	0.98 (0.84, 1.14)	0.93 (0.78, 1.12)	0.94 (0.78, 1.15)
OH-MeP	0.98 (0.87, 1.10)	0.98 (0.87, 1.11)	0.98 (0.86, 1.11)	1.02 (0.92, 1.13)	1.01 (0.91, 1.13)	1.01 (0.90, 1.13)
OH-EtP	1.05 (0.95, 1.17)	1.04 (0.95, 1.14)	1.04 (0.95, 1.13)	1.01 (0.98, 1.05)	1.01 (0.98, 1.05)	1.01 (0.97, 1.05)
TCS	1.01 (0.96, 1.06)	1.01 (0.96, 1.06)	1.01 (0.95, 1.06)	1.01 (0.95, 1.07)	1.00 (0.95, 1.06)	1.01 (0.94, 1.07)
TCC <sup>d</sup>	0.91 (0.62, 1.33)	0.95 (0.63, 1.41)	0.89 (0.56, 1.42)	0.98 (0.69, 1.38)	1.00 (0.70, 1.43)	1.03 (0.68, 1.58)

Abbreviations: FOR, Fecundability odds ratios; MP, methyl paraben; EP, ethyl paraben; PP, propyl paraben; BP, butyl paraben; BzP, benzyl paraben; 4-HB, 4-hydroxy benzoic acid; 3,4-DHB, 3,4-dihydroxy benzoic; OH-Me-P, methyl-protocatechuic acid; OH-Et-P, ethyl-protocatechuic acid; TCS, triclosan; TCC, triclocarban.

<sup>a</sup> Cox proportional odds models were adjusted for age, creatinine, BMI ( $25 \leq \text{BMI} < 30$ ,  $30 \leq \text{BMI} < 35$ , and  $\geq 35 \text{ kg/m}^2$  compared with  $\text{BMI} < 25 \text{ kg/m}^2$ ), smoking status (cotinine dichotomized at a threshold of 10 ng/mL), race/ethnicity (dichotomized, Non-White vs. White) and income (dichotomized at \$70,000).

<sup>b</sup> Cox proportional odds models were adjusted for female age, difference between partners' age, both partner's: creatinine, BMI ( $25 \leq \text{BMI} < 30$ ,  $30 \leq \text{BMI} < 35$ , and  $\geq 35 \text{ kg/m}^2$  compared with  $\text{BMI} < 25 \text{ kg/m}^2$ ), smoking status (cotinine dichotomized at a threshold of 10 ng/mL), race/ethnicity (dichotomized, Non-White vs. White), income (dichotomized at \$70,000) and partner's continuous concentrations of urinary chemicals.

<sup>c</sup> p-value < 0.05 prior to rounding.

<sup>d</sup> FOR and 95% CI reported are from models of dichotomous chemical concentrations (above/below LOQ).

**Figure 1.** Female partners' urinary MP and EP (in quartiles) and fecundability odds ratios.

**Footnotes:** Abbreviations: FOR, fecundability odds ratio; MP, methyl paraben; EP, ethyl paraben.

Error bars represent 95% CI confidence intervals. FORs are from unadjusted Cox proportional odds models.

aFORs were adjusted for age, creatinine, BMI ( $25 \leq \text{BMI} < 30$ ,  $30 \leq \text{BMI} < 35$ , and  $\geq 35 \text{ kg/m}^2$  compared with  $\text{BMI} < 25 \text{ kg/m}^2$ ), smoking status (cotinine dichotomized at a threshold of 10 ng/mL), race/ethnicity (dichotomized, Non-White vs. White) and income (dichotomized at \$70,000).

**Figure 2.** Female partners' urinary MP and EP and fecundability odds ratios (couple-based analysis).

**Footnotes:** Abbreviations: FOR, fecundability odds ratio; MP, methyl paraben; EP, ethyl paraben.

Error bars represent 95% CI confidence intervals. FORs are from unadjusted Cox proportional odds models of both female and male partners' urinary chemical concentrations. aFORs are from Cox proportional odds models adjusted for female age, difference between partners' age, both partner's: creatinine, BMI ( $25 \leq \text{BMI} < 30$ ,  $30 \leq \text{BMI} < 35$ , and  $\geq 35 \text{ kg/m}^2$  compared with  $\text{BMI} < 25 \text{ kg/m}^2$ ), smoking status (cotinine dichotomized at a threshold of 10 ng/mL), race/ethnicity (dichotomized, Non-White vs. White), income (dichotomized at \$70,000) and urinary chemical concentrations.

Figure 1.

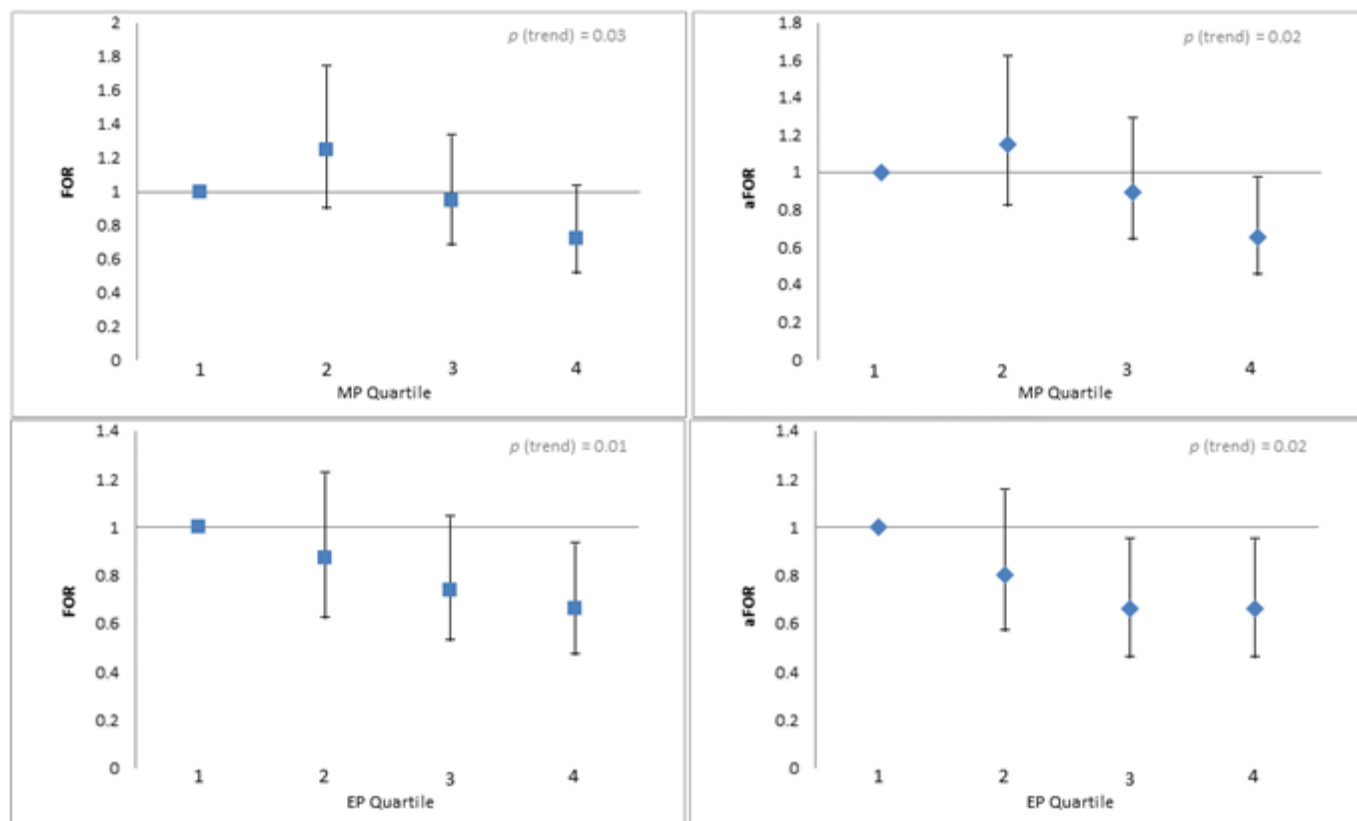


Figure 2.

